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Biophysical Chemistry 134 (2008) 39–46

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

The psychotropic drug olanzapine (Zyprexa®) increases the area of acid glycerophospholipid monolayers

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Received 16 October 2007; received in revised form 7 January 2008; accepted 8 January 2008
Available online 17 January 2008

Abstract

The typical antipsychotics chlorpromazine (CPZ) and trifluoperazine (TFP) increase the mean molecular area (mma) of acidic, but not neutral, glycerophospholipids in monolayers at pH 7.36 measured by the Langmuir technique. The atypical antipsychotic olanzapine (OLP [1]) is structurally similar to TFP. We have therefore studied the effects of OLP on glycerophospholipid monolayers and in comparison with CPZ. Olanzapine (10 μM, in subphase, pH 7.36) influenced the isotherms (surface pressure versus mma) in monolayers of the neutral dipalmitoyl phosphatidylcholine (DPPC) and the acidic dipalmitoyl phosphatidylserine (DPPS) or 1-palmitoyl-2-oleoylphosphatidylserine (POPS) in the increasing order of mma: DPPS < DPPC < POPS at both lower and higher temperature. Thus, presence of an unsaturated acyl in PS increased the drug-induced effect on mma. The mma in the absence of drugs was lower at lower temperatures than at higher temperatures. OLP affected mma to a greater extent than CPZ, and caused the greatest interaction at surface pressure of 30 mN/m at higher temperatures. In contrast, CPZ gave the largest effect in the monolayers at surface pressure 30 mN/m at lower temperatures. CPZ did not alter the isotherms of DPPC, at lower or higher temperature, and only affected the packing of the DPPS and POPS monolayers. In contrast, OLP altered the isotherms of DPPC. It is suggested that the drugs affect the monolayer packing by intercalating between the glycerophospholipid molecules. Since CPZ has major side effects, while OLP has few, this may indicate that there is poor correlation between side effects and effects of the drugs on phospholipid monolayers.

Keywords: Olanzapine; Chlorpromazine; Dipalmitoylphosphatidylcholine; Dipalmitoylphosphatidylserine; 1-palmitoyl-2-oleoylphosphatidylserine; Langmuirisotherm

1. Introduction

The classical psychotropic drugs chlorpromazine (CPZ) and trifluoperazine (TFP) interfere with polyphosphoinositide (PPI) metabolism in stimulated platelets [1–5]. These cells do not have D_2 receptors [6], which are assumed to be the main target for the phenothiazines [7,8], and the interference was thought to be non-receptor-mediated [9].

In micromolar concentrations CPZ [10–14] and TFP [13,15] cause large increases in the mean molecular areas in monolayers of acidic but not of neutral glycerophospholipids. Recent ^{31}P- and ^{13}C NMR studies [16–18] showed that CPZ interacts poorly with liposomes with neutral glycerophospholipids, while it causes a large shift of ~30% of acyl chain carbon resonances in liposomes with the negatively charged phosphatidylserine (PS) from pig brain. Specifically, the positively charged aliphatic N of CPZ (see Scheme 1) was shown to bind electrostatically to the negatively charged phosphate group in PS and presence of docosahexenoic in PS increased the binding of CPZ to the liposomes [19]. Interaction of CPZ with acidic, but not with neutral glycerophospholipid bilayer vesicles has also been

Abbreviations: CPZ, chlorpromazine; DPPC, dipalmitoyl phosphatidylcholine; DPPS, dipalmitoyl phosphatidylserine; OLP, olanzapine; POPS, 1-palmitoyl-2-oleoyl phosphatidylserine; PPI, polyphosphoinositide; PS, phosphatidylserine; TFP, trifluoperazine.

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[1] The uncharged and positively charged forms of OLP are designated OLP^0 and OLP^+, respectively.

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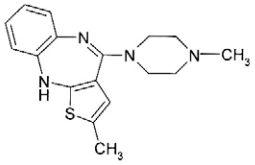
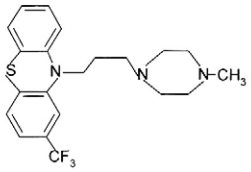
doi:10.1016/j.bpc.2008.01.003



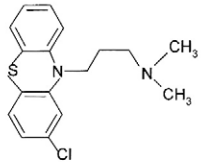
Olanzapine

{(2-Methyl-4-(4-methyl-1-piperazinyl)10H-thieno[2,3b][1,5]benzodiazepine}



Trifluoperazine

{10-[3-(4-methyl-1-piperazinyl)-propyl]-2-trifluoromethyl-10H-phenothiazine}



Chlorpromazine

{2-chloro-N,N-diethyl-10H-phenothiazine-10-propanamine}

Scheme 1. Structure formulaes of olanzapine, trifluoperazine and chlorpromazine.

shown by fluorescence [20]. These physicochemical observations strongly suggest that CPZ *intercalates* among PS molecules in the membranes with CPZ's headgroup interacting electrostatically to the polar part and the hydrophobic phenothiazine group immersed among the acyl groups of PS. This intercalation alters the structure of the PS membrane. Therefore, it was suggested that the phenothiazines affected PPI metabolism in platelets through intercalation in the cell membranes that altered the relative positions of the inositol lipid substrates to their enzymes in the PPI cycle, a phenomenon referred to as "alteration in substrate availability" [9].

Both TFP and CPZ are amphiphilic molecules with hydrophobic phenothiazine ring systems and positively charged (hydrophilic) tail groups at pH 7.4, an aliphatic tail for CPZ and a piperazine group for TFP (Scheme 1). However, although both phenothiazines have excellent antipsychotic properties, their severe side effects (extrapyramidal effects such as parkinsonism, akathasia, and dystonia besides drowsiness, dizziness, blurred vision, dry mouth, upset stomach and more) have caused an intense search the last 25 years for new ("atypical") antipsychotic drugs with far less side effects. One of these new drugs is olanzapine (OLP), which like the phenothiazines also antagonizes D_2 receptors [21,22], but OLP antagonizes serotonergic ($5HT_{2A}$, $5HT_{2C}$), adrenergic (α_1), muscarinic, histaminergic (H_1) [21] and nicotinic [23] receptors as well. Olanzapine also exerts non-neuroleptic effects, such as inhibition of amino acid transport in fibroblasts [24] and increase in serum creatine kinase levels [25].

With a structure resembling that of TFP (Scheme 1), OLP should display amphiphilic properties and be ionizable at high pH, and thus likely to be intercalated in acidic phospholipid membranes. Like TFP, OLP has two ionizable nitrogens in the piperazine ring with a p*K*s of 7.4 and 4.7 [26] suggesting that OLP is partly positively charged at physiological pH. However, if OLP intercalates among anionic glycerophospholipids as described for CPZ above, the dissociation reaction $OLP^0 + H^+ \leftrightarrow OLP^+$, would be driven far to the right as OLP^+ would be trapped by intercalation in the membrane. Conversely, if OLP binds to neutral phospholipids in the neutral form, the reaction would be driven to the left as OLP^0 would be trapped in the hydrophobic acyl layer of the membrane.

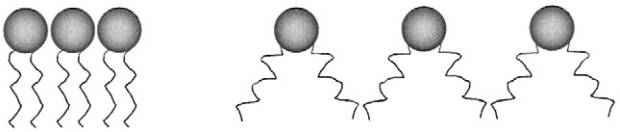
The partition of OLP between octanol and water is reported to be 50 [26]. However, octanol is obviously not a representative model for the hydrophobic milieu in biological membranes. For example, the partition of TFP between microsomes, liposomes, erythrocyte ghosts or octanol and water has been reported to be 7172, 1916, 1380 and 452, respectively [27]. Moreover, partition of CPZ between water and biological membranes has been reported with coefficients ranging from 850 to 1700, depending both on the temperature and the length of the glycerophospholipid acyl chains and their degree of unsaturation [28], and the partition of TFP into liposomes containing PS has reported to have coefficients in the order of $6.0–6.6 \times 10^5$ [29].

It is, therefore, likely that OLP also will be intercalated in monolayer membranes of negatively charged glycerophospholipids. Earlier study of TFP [15] and the Langmuir experiments on CPZ are used as positive controls. We report here results from studies with the Langmuir monolayer technique of the interaction between OLP and certain glycerophospholipids and in comparison with CPZ. The interaction between the two psychotropics and the lipids have been studied both at lower and higher temperatures.

2. Materials and methods

2.1. Lipids and chemicals

Dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylserine (DPPS) and 1-palmitoyl-2-oleoylphosphatidylserine (POPS) were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). The lipids were kept in the dark as powders or chloroform solutions at −20 °C. Chlorpromazine (CPZ) was from Sigma Chemical Co. (St. Louis, MO), and olanzapine (OLP) was kindly provided by Eli Lilly and Company (Indianapolis, IN). Stock solutions (10 mM) of CPZ and OLP



Scheme 2.

Table 1
Sample temperatures of the glycerophospholipids used in the Langmuir monolayer isotherm experiments

Glycerophspholipid	DPPC	DPPS	POPS
Lower sample temperature, °C	37	37	8
Higher sample temperature °C	43	58	37

were made in 0.9% NaCl and in ethanol, respectively. Working solutions were made by diluting the stock solutions with HEPES buffer in a ratio of 1:1000 (v/v). HEPES buffer (5 mM, pH 7.36) was purchased from Sigma-Aldrich. All other chemicals were of analytical grade. MilliQ water with low ionic concentration (18.2 MΩ/cm was obtained by an instrument (Academic) from Millipore.

2.2. Experimental

A KSV Minitrough (Helsinki, Finland) of dimensions 75 (w)×364 (l)×5 (h) mm was used in the study of the monolayer at the air/water interface. The procedure and explanation of the compression phases are described elsewhere [15,30]. The trough was filled with HEPES buffer (10 mM, pH=7.36) with and without 10 μM drugs and without alkaline cations. Experiments were carried out using a thermostatted bath at temperatures both at lower and higher temperatures. During the experiments, the samples were kept at the desired temperature ±0.5 °C. The experimental temperatures are listed in Table 1. In each experiment 10 μl of glycerophospholipid dissolved in chloroform (1 mg/ml) was carefully spread on the aqueous surface with a Hamilton syringe, and the chloroform allowed to evaporate before the compression started. During compression, the barrier speed was kept at 5 mm/min and the subphase thermostatted as noted. The surface pressure, Π, was determined using the Wilhelmy plate method. The lift-off areas were defined the mma when the surface pressure has reached 1 mN/m.

The experiments were usually performed with the amphiphilic drug dissolved in H_2O. OLP is not soluble in water, and was therefore dissolved in ethanol. Isotherms for all glycerophospholipids were also obtained by adding 1 ml ethanol in 1 l Hepes buffer solution. No difference in the isotherms was found for any of the phospholipids between the phospholipids with or without appropriate amounts of ethanol.

2.3. Statistics

All experiments were repeated three times, and SDs were calculated by the Excel or Sigma plot programs.

3. Results

At pH=7.36 CPZ is positively charged. OLP has a p*K*a value around 7.4, and there are two OLP species at pH=7.36, one neutral and one positively charged. The structure of CPZ and OLP are shown in Scheme 1.

3.1. Results from running the isotherms at lower and higher temperatures

The effects on the mean molecular area upon changing the sample temperature from the lower to the higher temperatures in the absence of drugs are shown in Table 2 and Fig. 1. At the lower temperatures the fluidity of the membrane is reduced compared to the higher temperatures and the corresponding mean molecular area is decreased (see Scheme 2). As indicated in Fig. 1, the mean molecular areas for the phospholipids of this study increase in the following order: DPPS<DPPC<POPS, both at the lower and the higher temperature. Furthermore, the mean molecular area is determined by both the kind of headgroups and acyl chain composition, that is saturation and the degree of unsaturation, in the investigated glycerophospholipids. Serine (PS) headgroup has −1 charge and is smaller than the electrical neutral choline (PC) headgroup. The results (Table 1) indicate that the temperature, the charge and size of the headgroup and the saturation of the acyl chains all contribute to the observed mean molecular area.

3.2. Results on neutral monolayer of DPPC upon adding CPZ and OLP to the subphase

The percent change in mean molecular area of DPPC upon adding CPZ and OLP at lower temperature is shown in Table 2. OLP displays the largest change in mean molecular area at lift-off for DPPC. CPZ, on the other hand, shows a reduction in mean molecular area at lift-off. However, the change in molecular area are lower for both CPZ and OLP at 30 mN/m, than at lift-off. The isotherms of DPPC with OLP and CPZ, show that OLP did affect the mean molecular area to a larger extent than CPZ. The results of DPPC upon adding CPZ and OLP at lower and higher temperatures are shown in Fig. 2. Both drugs increase the mean molecular area. The change in molecular area at lift-off were 18% and 8% for OLP or CPZ is present in the subphase, respectively. At 30 mN/m, the change in molecular area were lower for both drugs than at lift-off. The observed isotherm of CPZ shown in Fig. 2 demonstrates that

Table 2
Mean molecular area in Å^2 for DPPC, DPPS and POPS without drugs at lift-off and at 30 mN/m are shown

	T	DPPC		DPPS		POPS	
		Lift-off (Å^2)	30 mN/m (Å^2)	Lift-off (Å^2)	30 mN/m (Å^2)	Lift-off (Å^2)	30 mN/m (Å^2)
	Higher	161	126.4±0.4	148	119.1±0.3	184	136.5±0.8
	Lower	158	127.8±0.7	139	120.1±0.3	162	135±1
CPZ	Higher	8	−0.2	11	8.1	10	7.3
(%)	Lower	0	−2.9	25	14.3	24	10.4
OLP	Higher	18	5.8	31	21.9	9	10.1
(%)	Lower	18	1.9	41	26.4	18	8.6

The lift-off areas were defined the mma when the surface pressure has reached 1 mN/m. The SDs at 30 mN/m are included in the table. Increase in % mean molecular area change at lift-off and at 30 mN/m upon adding drugs at lower and higher temperatures are also shown.

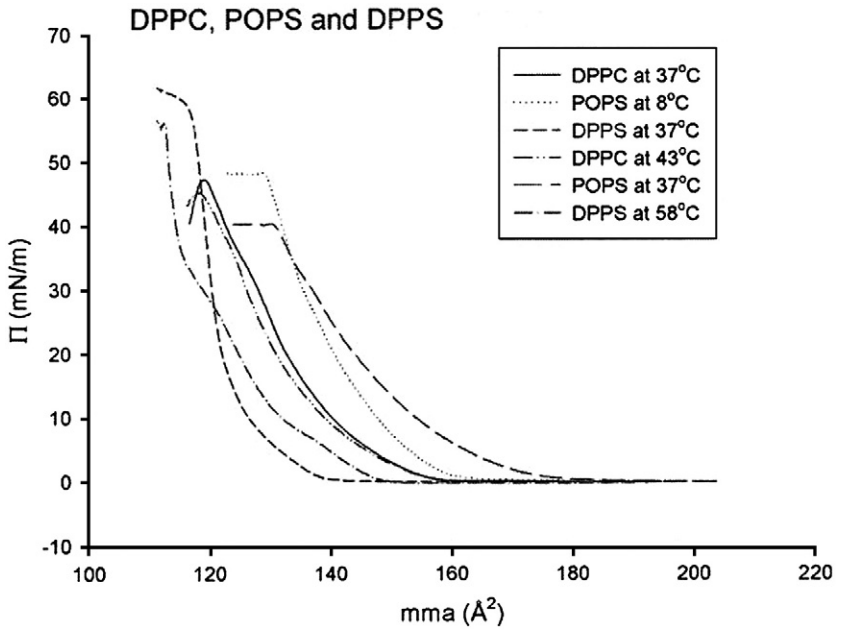


Fig. 1. The isotherms of the lipids at both the lower and the higher temperatures.

this isotherm is almost identical to the isotherms obtained without CPZ in the subphase. The results of adding CPZ or OLP in the subphase show that CPZ has only a minor effect on the neutral monolayer of DPPC, in contrast to OLP, which has a large effect on the monolayer packing of DPPC.

3.3. Results on monolayer of negatively charged DPPS upon adding CPZ and OLP in the subphase

DPPS has net charge of −1. The isotherms of DPPS with and without drugs at the higher temperature are shown in Fig. 3. The percent increase in mean molecular area at lift-off with drugs present in the subphase at the higher temperature is shown in Table 2. The percent increases are 11% for CPZ and 31% for OLP. At 30 mN/m the increase were 8.1% for CPZ and 21.9% for OLP. OLP is affecting the packing of the DPPS monolayer to a larger extent than CPZ. Further, the isotherm with OLP in the subphase is showing a region where the surface pressure is constant before the isotherm increase and collapse. The collapse point is concurrent with the collapse point with DPPS without OLP added in the subphase. Fig. 3 includes the isotherms of DPPS at lower temperature. Both drugs are affecting the

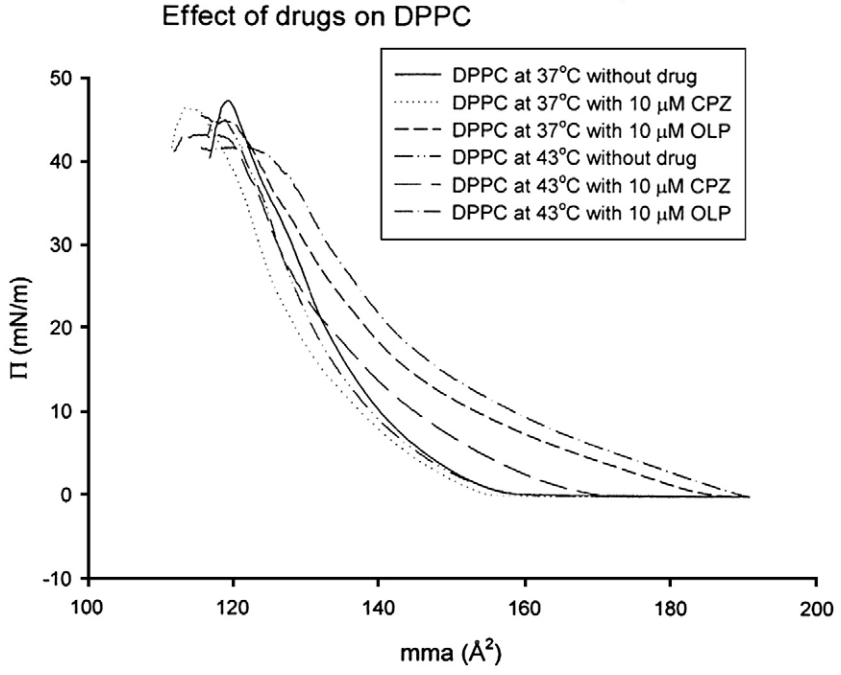


Fig. 2. The figure shows the isotherms of DPPC with and without drugs added in the subphase at both the lower and the higher temperature.

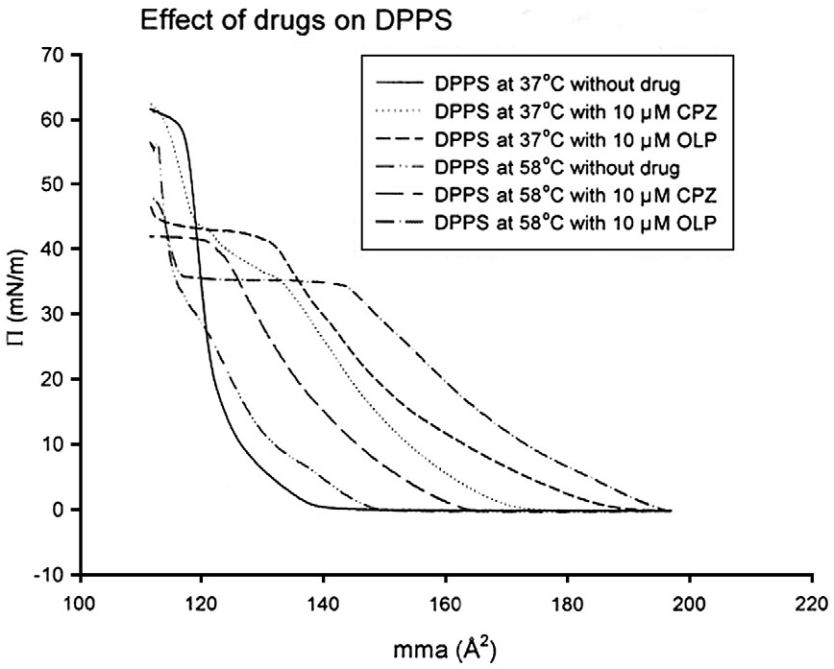


Fig. 3. The figure shows the isotherms of DPPS with and without drugs added in the subphase at both the lower and the higher temperature.

monolayer packing. At the lower temperature, both CPZ and OLP are affecting the monolayer packing. The isotherms are showing the same shape upon drug present in the subphase. However, OLP shows a more pronounced effect on the monolayer packing than CPZ at the higher temperature.

3.4. Results on monolayer of one unsaturated acyl chain by POPS upon adding CPZ and OLP in the subphase

POPS has a negatively charged headgroup at pH = 7.36 and the sn-2 acyl chain is unsaturated at carbon 9.The isotherms of POPS with CPZ or OLP in the subphase at the lower temperature and the increase in percent change in mean molecular area upon adding the drugs are shown in Table 2. The results show that both drugs change the mean molecular area, where CPZ shows the most pronounced effect on both the isotherms and an increase in mean molecular area. CPZ increased the mean molecular area at lift-off at the lower temperature of 24%, while OLP increased by 18%. At the lower temperature, at 30 mN/m, CPZ increased the mma by 10.4% and OLP increased by 8.6%.This observation, that CPZ had higher effect on the monolayer than OLP is only observed on POPS at the lower temperature. The isotherms of POPS upon adding the drugs at the lower and the higher temperature are shown in Fig. 4. OLP shows more pronounced effect on the monolayer packing than CPZ. The percent increase in the mean molecular area at lift-off were 10% and 9% for CPZ and OLP, respectively. At 30 mN/m, the changes in mean molecular area were 7.3% for CPZ and 10.1% for OLP. The results show that OLP has more effect on the monolayer packing at the higher temperature than CPZ. Both DPPS and POPS have a negatively charged headgroup. The observed differences in the isotherms of these two monolayers might be due to the difference in the acyl chains of the two lipids. DPPS has two saturated acyl chains, while POPS has one saturated and one monounsaturated acyl chain. As shown in Fig. 4, the effects of OLP or CPZ in the subphase were lower for POPS than for DPPS. Table 2 shows that the mean molecular area were less both at lift-off and at 30 mN/m for DPPS. However, Fig. 4 and Table 2 show that POPS without drugs added in the subphase, has a higher molecular area than DPPS without drugs both at lift-off and at 30 mN/m. This indicates that POPS with an unsaturated acyl chain is affecting the monolayer more than a lipid with both acyl chains saturated. OLP was solved in ethanol. To ensure that the observed changes in mean molecular area was not due to the presence of ethanol, experiments with only ethanol added in the subphase where done on all three monolayers of the phospholipid. The resulting isotherms were not affected by ethanol (data not shown).

4. Discussion

4.1. Effect of temperature on lipid monolayers

The Langmuir monolayer technique revealed that the mean molecular area of the phospholipids without drugs increased in the order: DPPS < DPPC < POPS at both the lower and the higher temperatures. Further, the mean molecular area was lower at the lower temperatures than at the higher temperatures for each lipid. The phospholipids occupy larger mean molecular area at higher temperatures corresponding to increased fluidity of the lipid monolayer. The isotherms show that POPS occupies the largest mean molecular area both at the lower and the higher temperature. This observation corresponds to a looser packing of the phospholipid monolayer caused by the unsaturated acyl chain present in POPS. The isotherms of the phospholipids with saturated acyl

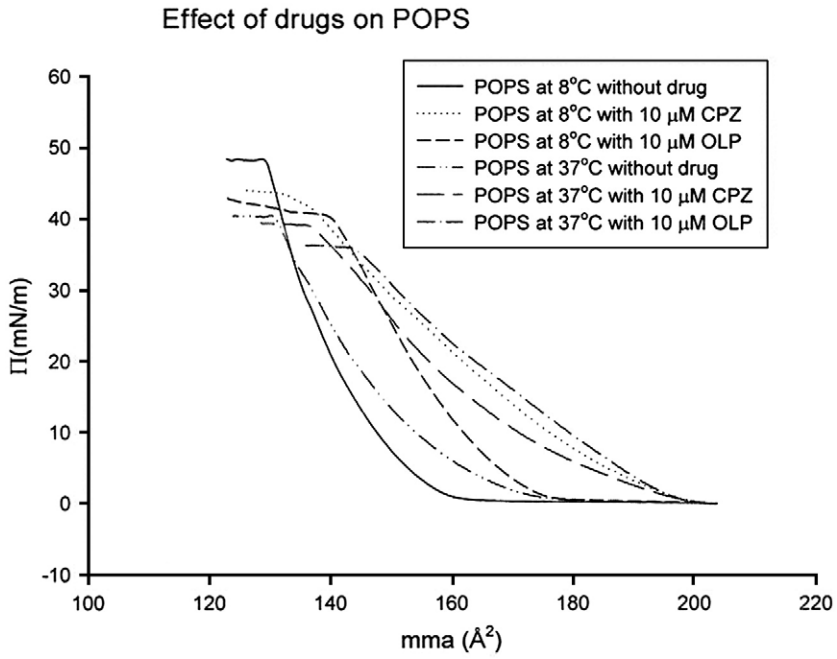


Fig. 4. The figure shows the isotherms of POPS with and without drugs added in the subphase at both the lower and the higher temperature.

chains show that DPPC is occupying the largest mean molecular area. The size of the headgroup in DPPC is bigger that the DPPS headgroup. A larger headgroup causes the acyl chain to be more separated, making the packing of the phospholipid monolayer looser. The acyl chains in DPPS are more closely packed, so the monolayer of DPPC collapses at lower surface pressure. Further, the sodium ions of the HEPES buffer might bind to the serine group in DPPS, so that the phospholipid becomes neutralized. The binding between the sodium ion and serine will stabilize the electrostatic repellent forces between the positive charged DPPS headgroups. Possible hydrogen bonds between serine and water molecules in the subphase might also contribute to a more rigid packing of the DPPS monolayer.

The results show that the temperature affects the monolayer fluidity, in that the packing of the monolayer is closer at lower than at higher temperatures. It is possible that at the lower temperature without no drug added, a phase transition and/or a domain formation occurs. This might explain why all the isothermes of the monolayers are shiftet to the left in Fig. 1.

4.2. Effect on neutral monolayer of DPPC

The results show that OLP affects the DPPC molecular area to a much larger extent than CPZ. In addition, OLP demonstrates the most pronounced interaction with DPPC at a surface pressure of 30 mN/m at the higher temperature. In contrast to that, CPZ hardly affects the neutral DPPC, neither at the lower nor at the higher temperature.

These observations are in accordance with results from earlier studies which demonstrates that CPZ only affects the packing of the monolayer of charged head groups [17]. It has also been demonstrated by solid state NMR that CPZ interdigitates between the acyl chains of the phospholipids [16]. At pH = 7.36, OLP will be present as two species, one positively charged, and one neutral. Since CPZ is positively charged, and had only minor effect on DPPC monolayer packing, we assume that it is not the charged specie of OLP that has the major contribution in the interference with the monolayer. We suggest that the neutral OLP molecules, (OLP^0) specie interfere with the DPPC monolayer packing by better intercalating in the monolayer. Thus, it is likely that the OLP^0 specie interacts with the DPPC monolayer by hydrophobic forces, and that the OLP^0 is intercalating in between the hydrophobic acyl chains of DPPC.

Intercalating OLP^0 will increase the mean molecular area of the lipid. Further, the interaction between OLP^0 and DPPC is even larger at the higher temperature. This observation supports the notion that the interaction between OLP^0 and the monolayer of DPPC is mainly hydrophobic. At the higher temperature, the lipid molecular area increases and OLP^0 intercalation will be enhanced (Fig. 5, right).

4.3. Effect on charged monolayer of DPPS

DPPS has a net charge of −1, and the positively charged CPZ ion might interact electrostatically to the negatively charged headgroup of DPPS [18]. The isotherms of DPPS with and without drugs showed that CPZ increased the mean molecular area, and has a large influence of the packing of the DPPS monolayer. The isotherms of CPZ with DPPS show that CPZ has the largest effect on the monolayer packing at the lower temperature. In fact, at the lower temperature the isotherm with CPZ is close to the OLP isotherm. Further, both isotherms with drug show a plateau where the mean molecular area is constant. This plateau indicate that both drugs are expelled from the interface at surface pressures below the monolayer collapse.

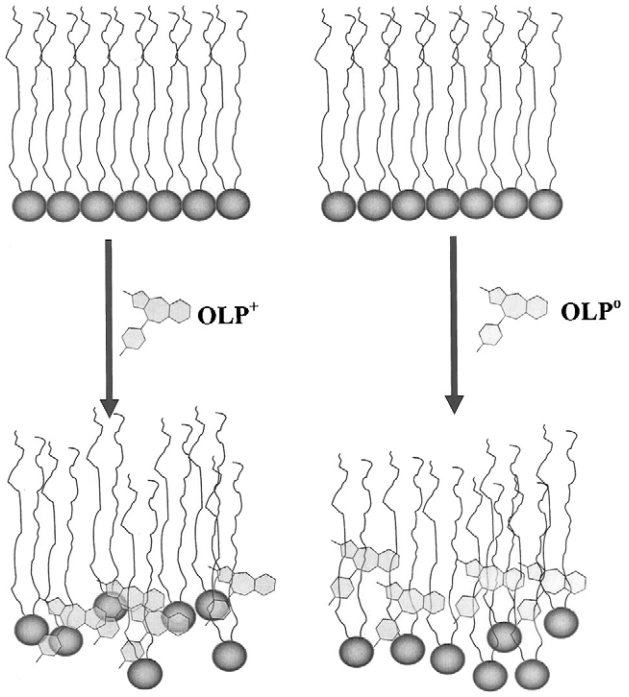


Fig. 5. Model of intercalation of OLP at pH=7.36 in a monolayer of negatively charged PS. The positive specie of OLP is interfering with the negatively charged headgroup of the lipid, (left) while the neutral specie is intercalated in between the acyl chains of the lipid (right).

The plateau is more pronounced with OLP in the subphase, and there is no plateau with CPZ in the subphase at the higher temperature of DPPS. However, OLP shows a larger effect on the lipid monolayer packing. The effect is very pronounced at the higher temperature.

This might indicate that both the charged headgroup in DPPS and the acyl chain separation both contribute to the interaction of OLP on the DPPS monolayer. Further, both OLP, OLP^0 and OLP^+ species might be involved in the phospholipid interaction, indicating that both electrostatic and hydrophobic interactions contribute.

The substantial effect of OLP on the monolayer packing of DPPS might be explained by the interaction of both species of OLP, OLP^0 and OLP^+ at the same time, in that both species are intercalating in the DPPS monolayer. The positively charged specie is involved with the negatively charged headgroup, and as a result is intercalating in the acyl chains of DPPS. The neutral species is interacting with the acyl chains of DPPS.

4.4. The effects on unsaturated acyl chain

POPS has a negatively charged headgroup and one acyl chain is mono-unsaturated. The effect of CPZ on the packing of POPS monolayer is more pronounced than the effect of OLP at the lower temperature. Conversely, at the higher temperature the effect of OLP on the molecular packing in the POPS monolayer is larger than at the lower temperature. However, both OLP and CPZ have stronger interaction with DPPS than with POPS. In the POPS monolayer the glycerophospholipids occupies a larger mean molecular area than the phospholipids in the DPPS monolayer due to the unsaturated acyl chain in POPS. The intercalation of the drugs is less in the monolayer of POPS compared with DPPS monolayer. On the other hand, the phospholipid interaction of these drugs is significant, and OLP is shown to have a larger influence on the monolayer of POPS than CPZ at the higher temperature. From this observation, we suggest that both species of OLP are interacting with the POPS monolayer, and that both the headgroup and the acyl chains of the phospholipids are involved in the interaction with OLP and CPZ. In Fig. 5 we suggest that the neutral OLP specie is better intercalated in the acyl chains. The charged species is also intercalated in the lipid monolayer, but the negative charge of the headgroup will react electrostatic with the positive charge of OLP. Thus, the neutral species of OLP will be better intercalated in the lipid monolayer. The results indicate that OLP intercalates both with acidic and neutral glycerophopholipids, while CPZ intercalates only between acidic glycerophospholipids. Recently, the interaction between OLP and DPPC and POPS have been studied by solid-state NMR and these results demonstrate that the drug intercalates in liposomes made of neutral DPPC and acidic POPS [31]. This is in good agreement with our results.

CPZ has major clinical side effects, while OLP has few, suggesting that there is no connection of the side effects and the effect observed on the phospholipid monolayer. This indicates that the interaction between the amphiphilic drugs and the membrane may correspond to the pshycotropic effect.

To our surprise, we observed little difference in the percent increase of the mean molecular area by the drugs at the higher or the lower temperatures of the phospholipids. We are not able to explain this observation, as one should expect more effect of the drugs at the higher temperature. However, without drugs present, there was a clear difference in mean molecular area at the higher and the lower temperature.

Acknowledgement

The work was supported by a grant from the Blix Foundation.

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